A COMPARATIVE STUDY OF THE LIPIDS IN SOME MARINE ANNELIDS

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Introduction

In the past, studies on the metabolism of lipids have been confined chiefly to observations made on homoiothermal vertebrates. Very little work has been done on the problem in the invertebrates.

Terroine and co-workers (1930) found that the phospholipids in various cold-blooded animals contain very low amounts of fatty acid, that the iodine number of the fatty acids is not constant, and that the iodine number of the phospholipid fatty acids is lower than that of the stored fat but higher than that in similar tissues of homoiothermes.

Timon-David (1930) found that the larvae of 24 insects contained 0.94–28 per cent fat and that linolenic and oleic acids were widely distributed.

Slifer (1932) found that the iodine number of the fatty acids in the eggs of seven species of grasshopper varies from 128–167. She found also that the temperature for complete fusion of the fatty acids depends on the environmental temperature: fats from eggs so laid as to hatch in spring had a melting point decidedly lower than fats from eggs so laid as to hatch in the summer.

Salisbury and Anderson (1939) found that the lipids in *Cysticercus fasciolaris* consist of phospholipids, cholesterol, cerebrosides, and a small amount of neutral fat; that the phospholipids make up about 30 per cent of the total lipids; and that the fatty acids contain saturated and unsaturated forms in the ratio of 1 : 1.

Wilber (1947) showed that starvation decreases the total lipid in the entire worm *Phascolosoma,* causes little change in the muscle lipids, and decreases markedly the fatty acid and phospholipid content of the coelomic fluid, but does not change the cholesterol content of the latter.

It is evident that information concerning lipid content and metabolism in the invertebrates is quite meager. A detailed study of the problem seems justified. The present report is one in a series dealing with the amounts and use of lipids in invertebrate tissues.

Material and Methods

Marine annelids were chosen for the investigation because they are obtainable readily and are easy to handle. The following species were studied: *Nereis pelagica, Amphitrite ornata, Arenicola marina, Phascolosoma gouldii, Lepidonotus squamatus, Glycera americana, Chaetopterus variopedatus.* All were procured in the living condition from the Supply Department of The Marine Biological Laboratory during the months of July and August, 1946.

Whole worms or individual tissues were prepared by grinding weighed amounts with sand in a mortar (Bloor, 1929). Lipids were then extracted with boiling alcohol. Some tissues were homogenized in a Waring-blendor instead of grinding with sand. The results from both methods are comparable.

Phospholipids were precipitated with acetone and magnesium chloride and estimated by the oxidation-titration method (Bloor, 1929). Fatty acid and cholesterol were estimated respectively by the oxidation-titration method and by the colorimeter using the acetic anhydride-sulfuric acid reagent (Bloor, 1928). This method measures the total fatty acids in the lipids.

Coelomic fluid was removed from some of the worms and the lipids were extracted and estimated in a similar manner.

From the results obtained, the lipocytic coefficients (cholesterol/fatty acid) and the ratio, cholesterol/phospholipid were calculated for the various tissues (Mayer and Schaeffer, 1913).

Results

The results are summarized in Table I which shows that there is a wide variation in the absolute values of the various lipid constituents in the different species.

Table I

Table showing the amounts of various lipids (values given as per cent of wet weight) in marine annelids and the numerical ratios of the lipids one to another. Values are the mean of ten worms selected at random

Species	Phospho-lipids	Choles-terol	Fatty acid	$\frac{\text{Chol.}}{\text{F.A.}}$	$\frac{\text{Ch.}}{\text{Ph.}}$	Total lipid
Nereis pelagica	0.73	0.35	1.82	0.19	0.48	2.17
Glycera americana	0.82	0.50	2.25	0.22	0.61	2.75
Amphitrite ornata	0.83	0.55	2.60	0.21	0.66	3.15
Arenicola marina	0.35	0.29	0.93	0.31	0.84	1.22
*Coelomic fluid**	155	55	877	0.06	0.35	932
Chaetopterus variopedatus	0.33	0.16	0.62	0.26	0.48	0.73
Lepidonotus squamatus	0.23	0.40	3.28	0.12	2.0	3.68

* Values in mg./100 cc. of coelomic fluid.

The table shows also that tissues with a high content of phospholipid have a high content of cholesterol. A similar relationship is apparent between cholesterol and fatty acid. If the values for fatty acid are plotted against the values for cholesterol, the points are distributed along a straight line.

Discussion

The results indicate that although there is a wide variation in the absolute values of phospholipid, fatty acid, and cholesterol in the different annelids, there is an apparently consistent ratio of these lipids one to another.

The phospholipids of the annelids studied make up about 37 per cent of the total lipid. This value is close to that obtained (30 per cent) by Salisbury and Anderson (1939) in *Cysticercus fasciolaris*.

It is known that the total lipid in the insect *Sphanarium purpurascens* consists mainly of free fatty acids (Giral, 1946). In the present investigation there is evidence of a predominance of fatty acid over cholesterol and phospholipid in annelid tissue.

The relationship between cholesterol and phospholipids is interesting. The worms with large amounts of cholesterol have a correspondingly large amount of phospholipid. A similar relationship obtains between cholesterol and fatty acid. This may indicate that in the annelids, as in the vertebrates, the ratio of lipids one to another is characteristic of the organ of an animal in a given species (Mayer and Schaeffer, 1913).

The results in general indicate that in the marine annelids, cholesterol is always associated with phospholipids and is in constant relation to them. Cholesterol is, therefore, probably a normal protoplasmic constituent in the annelids as in the vertebrates (Bloor, 1943).

Whether these relationships are constant under different environmental conditions (changes in temperature, salt concentration, or season) is not known at present.

Summary

1. The amount of cholesterol, fatty acid, and phospholipid in various marine annelids was estimated.

2. It was found that worms with a high content of cholesterol have also large amounts of phospholipid and fatty acid.

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